

12/14/95

WHAT IS CLAIMED IS:

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1. An isolated nucleic acid fragment comprising a sequence of at least about 20 nucleotides from a *Brassicaceae* or *Helianthus* delta-12 fatty acid desaturase gene having at least one mutation, wherein said at least one mutation is effective for increasing levels of oleic acid in *Brassicaceae* or *Helianthus* seeds and wherein said sequence includes said at least one mutation.
 2. The nucleic acid fragment of claim 1, wherein said sequence comprises a full-length coding sequence of said gene.
 3. The nucleic acid fragment of claim 1, wherein said mutant desaturase gene encodes a microsomal gene product.
 4. The nucleic acid fragment of claim 1, wherein said at least one mutation comprises a mutation in a region of said desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif.
 5. The nucleic acid fragment of claim 4, wherein said at least one mutation comprises a non-conservative amino acid substitution in said region.
 6. The nucleic acid fragment of claim 5, wherein said motif comprises the sequence His-Glu-Cys-Gly-His.
 7. The nucleic acid fragment of claim 6, wherein said at least one mutation comprises the sequence His-Lys-Cys-Gly-His.

8. The nucleic acid fragment of claim 1, wherein said mutant desaturase gene is from a *Brassica napus* plant.

9. The nucleic acid fragment of claim 1, wherein said gene is the D form of a *Brassicaceae* microsomal gene.

5 10. The nucleic acid fragment of claim 1, wherein said at least at least one mutation comprises the sequence Lys-Tyr-His-Asn-Asn-Pro.

11. A *Brassicaceae* or *Helianthus* plant containing a sequence of at least 20 nucleotides from a delta-12 fatty
10 acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif and wherein said mutation confers an altered fatty acid composition in seeds of said plant.

12. The plant of claim 11, wherein said plant contains a
15 full-length coding sequence of said mutant gene.

13. The plant of claim 11, wherein said mutation confers a decreased linoleic acid level in said seeds.

14. The plant of claim 11, wherein said mutant desaturase gene encodes a microsomal gene product.

20 15. The plant of claim 11, wherein said at least one mutation comprises a non-conservative amino acid substitution in said region.

16. The plant of claim 15, wherein said motif comprises the sequence His-Glu-Cys-Gly-His.

17. The plant of claim 16, wherein said at least one mutation comprises the sequence His-Lys-Cys-Gly-His.

18. The plant of claim 17, designated by ATCC deposit number 40811 or 75446.

5 19. The plant of claim 11, wherein said gene is from a *Brassica napus* plant.

20. The plant of claim 11, wherein said plant is a *Brassica napus* plant.

21. The plant of claim 20, wherein said gene is the D
10 form of said gene.

22. The plant of claim 21, further comprising a second mutation, said second mutation in the F form of a delta-12 fatty acid desaturase gene.

23. The plant of claim 20, wherein said gene is the F
15 form of said gene.

24. The plant of claim 23, further comprising a second mutation, said second mutation in the D form of a delta-12 fatty acid desaturase gene.

25. The plant of claim 22, wherein said second mutation
20 is in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif.

26. The plant of claim 24, wherein said second mutation is in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif.

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27. An isolated nucleic acid fragment comprising a sequence of at least about 20 nucleotides from a *Brassicaceae* or *Helianthus* delta-15 fatty acid desaturase gene having at least one mutation, wherein said at least one mutation is effective for increasing levels of oleic acid in *Brassicaceae* or *Helianthus* seeds and wherein said sequence includes said at least one mutation.

28. The nucleic acid fragment of claim 27, wherein said sequence comprises a full-length coding sequence of said gene.

29. The nucleic acid fragment of claim 27, wherein said mutant desaturase gene encodes a microsomal gene product.

30. The nucleic acid fragment of claim 27, wherein said at least one mutation comprises a mutation in a region of said desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif.

31. The nucleic acid fragment of claim 30, wherein said at least one mutation comprises a non-conservative amino acid substitution in said region.

32. The nucleic acid fragment of claim 31, wherein said motif comprises the sequence His-Asp-Cys-Gly-His.

33. The nucleic acid fragment of claim 32, wherein said at least one mutation comprises the sequence His-Lys-Cys-Gly-His.

34. The nucleic acid fragment of claim 27, wherein said mutant desaturase gene is from a *Brassica napus* plant.

35. A *Brassicaceae* or *Helianthus* plant containing a sequence of at least 20 nucleotides from a delta-15 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif and wherein said mutation confers an altered fatty acid composition in seeds of said plant.

36. The plant of claim 35, wherein said plant contains a full-length coding sequence of said mutant gene.

37. The plant of claim 35, wherein said mutation confers a decreased level of α -linolenic acid in said seeds.

38. The plant of claim 35, wherein said mutant desaturase gene encodes a microsomal gene product.

39. The plant of claim 35, wherein said at least one mutation comprises a non-conservative amino acid substitution in said region.

40. The plant of claim 39, wherein said motif comprises the sequence His-Asp-Cys-Gly-His.

41. The plant of claim 40, wherein said at least one mutation comprises the sequence His-Lys-Cys-Gly-His.

42. The plant of claim 35, wherein said mutant desaturase gene is from a *Brassica napus* plant.

43. The plant of claim 35, wherein said plant is a *Brassica napus* plant.

44. A *Brassicaceae* or *Helianthus* plant containing:

a) a sequence of at least 20 nucleotides from a delta-12 fatty acid desaturase gene having at least one mutation, said at least one delta-12 gene mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif;

b) a sequence of at least 20 nucleotides from a delta-15 fatty acid desaturase gene having at least one mutation, said at least one delta-15 gene mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif; and

c) said delta-12 gene mutation and said delta-15 gene mutation conferring an altered fatty acid composition in seeds of said plant.

45. The plant of claim 44, wherein said mutant genes confer a decreased level of α -linolenic acid in said seeds.

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46. A *Brassicaceae* or *Helianthus* plant containing a sequence of at least 20 nucleotides from a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a Tyr-Leu-Asn-Asn-Pro amino acid motif and wherein said mutation confers an altered fatty acid composition in seeds of said plant.

47. A vegetable oil extracted from seeds produced by the plant of claim 11.

48. The oil of claim 47, wherein said oil has, following crushing and extraction of said seeds, from about 1.0% to about 10.0% linoleic acid based on total fatty acid composition.

49. The oil of claim 47, wherein said oil has, following crushing and extraction of said seeds, from about 69% to about 90% oleic acid based on total fatty acid composition.

50. The oil of claim 49, wherein said oil has from about
5 80% to about 90% oleic acid based on total fatty acid composition.

51. A vegetable oil extracted from seeds produced by the plant of claim 35.

52. The oil of claim 51, wherein said oil has, following
10 crushing and extraction of said seeds, from about 0.5% to about 10.0% α -linolenic acid based on total fatty acid composition.

53. A vegetable oil extracted from seeds produced by the plant of claim 44.

15 54. A vegetable oil extracted from seeds produced by the plant of claim 46.

Sub A1 55. A method for producing a *Brassicaceae* or *Helianthus* plant line, comprising the steps of:

- 20 a) inducing mutagenesis in cells of a starting variety of a *Brassicaceae* or *Helianthus* species;
b) obtaining progeny plants from said cells;
c) identifying at least one of said progeny plant that contains a delta-12 fatty acid desaturase gene having at least one mutation, said at least one
25 mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif;

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e) producing said plant line from said at least one progeny plant by self-pollination for at least three additional generations.

56. The method of claim 55, wherein said plant line
5 yields an oil having a stabilized linoleic acid content from about 2.0 % to about 12.0 %.

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57. The method of claim 55, further comprising the steps of:

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f) inducing mutagenesis in cells of said plant line;

h) obtaining progeny plants from said plant line cells;

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d) identifying at least one of said plant line progeny plants that contains a delta-15 fatty acid desaturase gene having at least one delta-15 gene mutation, said at least one delta-15 gene mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif;

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e) producing a second plant line from said at least one plant line progeny plant by self-pollination for at least three additional generations.

58. The method of claim 55, wherein said starting variety is a *Brassica napus* variety.

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59. The method of claim 58, wherein said mutation is in a first form of delta-12 fatty acid desaturase.

60. The method of claim 59, further comprising the step of crossing a plant of said plant line to a plant having a mutation in a second form of delta-12 fatty acid desaturase.

61. The method of claim 60, wherein said second mutation
5 is in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif.

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62. The method of claim 58, further comprising the steps
of:

- 10 f) inducing mutagenesis in cells of said plant line;
h) obtaining progeny plants from said plant line cells;
d) identifying at least one of said plant line
15 progeny plants that contains a second delta-12 fatty acid desaturase gene having at least one mutation, said second gene mutation in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif;
e) producing a second plant line from said at
20 least one plant line progeny plant by self-pollination for at least three additional generations.

63. The method of claim 55, wherein said identifying step comprises a technique selected from the group
25 consisting of: PCR, 3SR and direct polynucleotide sequencing.

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64. A method for producing a *Brassicaceae* or *Helianthus* plant line, comprising the steps of:

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- a) inducing mutagenesis in cells of a starting variety of a *Brassicaceae* or *Helianthus* species;
 - b) obtaining progeny plants from said cells;
 - c) identifying at least one of said progeny plants that contains a delta-15 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif;
 - d) producing said plant line from said at least one progeny plant by self-pollination for at least three additional generations.

65. The method of claim 64, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.